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Abundance of Food Affects Relative Size of Larval and Postlarval Structures of a Molluscan Veliger

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Abstract. Veliger larvae of mollusks were predicted to develop a larger velum relative to the larval shell when reared with scarce food. The functional consequences of such developmental plasticity would be (1) greater maximum capacity for capturing particles when food is scarce and (2) greater growth of structures retained in the postlarva when food is abundant. The hypothesis was tested by rearing veligers of the oyster *Crassostrea gigas* at high (near satiating) and low (growth limiting) concentrations of food. Veligers at the measured shell lengths (>200 μm) had significantly larger velar lobes and longer prototrochal cilia than veligers reared in low concentrations of food. An analogous response to food levels (relatively longer ciliated band when food is scarce) has now been found for larvae as disparate as oyster veligers and sea urchin plutei. These observations suggest that functionally similar examples of developmental plasticity in the growth of larval parts have evolved more than once and may be widespread. An alternative interpretation is that differential mortality or growth in a genetically heterogeneous batch of oyster larvae results in advanced veligers of different forms at different concentrations of food. Both interpretations suggest an adaptive advantage to growing a larger apparatus for clearing particles from suspension when food is scarce and shifting materials to growth of postlarval structures (shell and associated structures) when food is abundant.

Introduction

The bodies of many larvae are divided into (1) those parts that are useful at settlement or are retained through metamorphosis and (2) ephemeral larval parts that are not needed for settling and are resorbed at metamorphosis. The ephemeral parts commonly are structures for feeding or defense during a planktonic larval period. Structures for suspension feeding constitute a major part of the ephemeral larval body of many larval forms (Strathmann, 1978; McEdward, 1984; Nielsen, 1987). These structures provide a high maximum clearance rate (Strathmann, 1987b), which is the rate at which a volume of water is cleared of suspended food particles. When suitable food is scarce, a suspension-feeding larva requires a high clearance rate to obtain sufficient nutrition for rapid growth and development. When food is abundant, however, and a high clearance rate would accumulate more food than could be digested, larvae behaviorally reduce their clearance rates or reject many of the captured particles (Strathmann, 1987b). Thus when food is abundant, larvae have a greater capacity for clearing particles from suspension than is needed.

These observations indicate different functional requirements for adaptation of larvae to high or low concentrations of food. When food is scarce, nutritional intake depends on a high clearance rate, and development of a large ephemeral apparatus for capturing particles is advantageous. When food is abundant, such a large ephemeral apparatus is superfluous, and growth should instead be allocated to other structures: those for digestion of food, those for storage of nutrients, or those that will become useful at or after settlement (Strathmann *et al.*, 1992). Because larvae within a population may encounter greatly different concentrations of food, an invariant type of larval development would result in either disadvantageously low clearance rates when food is scarce or an inefficient diversion of growth to unused ephemeral structures when

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food is abundant. One solution to this functional problem is developmental plasticity.

Developmental plasticity of this kind is known for the echinoplutei of sea urchins. These larvae grow longer larval arms when food is scarce and shorter arms when food is abundant. The maximum clearance rate depends on the length of the ciliary band (Strathmann, 1987b; Hart, 1991), and longer arms bear longer ciliary bands. Also, when food is abundant, the development of rudiments of postlarval structures is accelerated relative to the external larval body (Boidron-Metairon, 1988; Hart and Scheibling, 1988; Strathmann *et al.,* 1992). These observations on echinoplutei led to the hypothesis that allocation of materials to structures used for larval feeding, as opposed to structures used at or after settlement, depends on the amount of suitable food available to larvae; but we know of no reports of this kind of developmental plasticity in other suspension-feeding larvae.

To test the generality of the above hypothesis, we have studied a larva of different form, feeding mechanism, and phylum, but with a similar functional problem. The veliger larvae of mollusks capture particles by different physical mechanisms than those used by the larvae of echinoderms (Strathmann, 1987b; Gallager, 1988). Mollusks and echinoderms are distantly related phyla, representing the spiralian and deuterostome lineages. Both molluscan veligers and echinoid plutei have ephemeral structures bearing ciliary bands that capture particles, and both have structures that endure through metamorphosis. In echinoplutei, the ciliary band and arms are only larval, but the echinus rudiment persists through metamorphosis. In veligers, the ciliary bands and velar lobes are ephemeral, but the shell and much of the remaining body persist through metamorphosis. We examined veligers of the oyster *Crassostrea gigas* for developmental plasticity of velar size relative to shell size.

Materials and Methods

Embryos were obtained from a spawning of nine female and two male oysters conditioned at 18°C at the Westcott Bay Sea Farm, which is north of Puget Sound at the north end of San Juan Island. Larvae were reared at the Friday Harbor Laboratories.

Embryos and larvae were maintained in jars, each containing 2 l of seawater that had been filtered through a 0.45 μm membrane filter. The initial concentration of D-stage larvae at first feeding was 1.1 ml^{-1}. Cultures were stirred mechanically by paddles pulled at 10 strokes min^{-1} (Strathmann, 1987a). The jars were maintained at 20.5 to 23.5°C, except when the water was changed every two days; the new water was briefly 17 to 20°C, and at least once was as low as 15°C.

At every water change, equal numbers of cells of the algae *Isochrysis galbana* (T-ISO) and *Chaetoceros calcitrans* were added to make a total concentration of 3000 cells ml^{-1} for low food levels and 30,000 cells ml^{-1} for high food levels. There were three replicate jars for each food treatment. The algal species and concentrations were selected on the basis of previous studies. *C. calcitrans* is superior to *I. galbana* as a food for the larvae of *C. gigas* (Waldock and Nascimento, 1979), but both support growth. Sprung (1984) found growth of veligers of *Mytilus edulis* fed *I. galbana* to be maximal at concentrations of 30,000 cells ml^{-1} and lower than the maximum at concentrations of 3000 cells ml^{-1}. Maximal growth of veligers of *Crassostrea virginica* required additions of algal food to more than 100,000 cells ml^{-1} in some experiments (Davis and Guillard, 1958; Rhodes and Landers, 1973), but the concentrations of veligers were greater in these studies than in ours, and therefore more algae may have been removed by grazing. Also, in these studies of *C. virginica,* the minimal additions of food required for maximal growth increased as the veligers developed, but the removal of algae by an increasing rate of larval grazing may be the cause, and the concentration required for the maximal growth of veligers of *Mytilus edulis* did not change (Sprung, 1984). A constant 30,000 cells ml^{-1} for the maximal ration was therefore considered adequate and less likely to introduce complications from overfeeding.

Because growth was variable, equal numbers of the largest larvae were taken for measurement from subsamples of equal volume from each jar.

Measurements of shell, velum, and velar cilia were taken from videotapes of larvae recorded through a compound microscope. All measurements were done with Image version 1.22 software by Wayne Rasband at NIH and were provided by the National Technical Information Service.

First, the length and width of the velum were recorded through the 4× objective as the larva swam up against a cover glass (Fig. 1A). We restricted the horizontal movements of larvae by placing them within a piece of nylon mesh with openings larger than their bodies. Because the velum is approximately elliptical, its circumference was approximated by $2\pi[((L/2)^2 + (W/2)^2)/2]^{1/2}$, in which L and W are the length and width of the velum.

Next, we measured the longest cilia that were observed on videotapes recorded through the 10× objective with DIC optics. These were the prototrochal cilia, recorded during their effective strokes as the larva swam upward against the cover glass; they were measured from the velar edge to their tips (Fig. 1C).

The shell was videorecorded and its length measured after the larva had been killed with a drop of 4% formalin

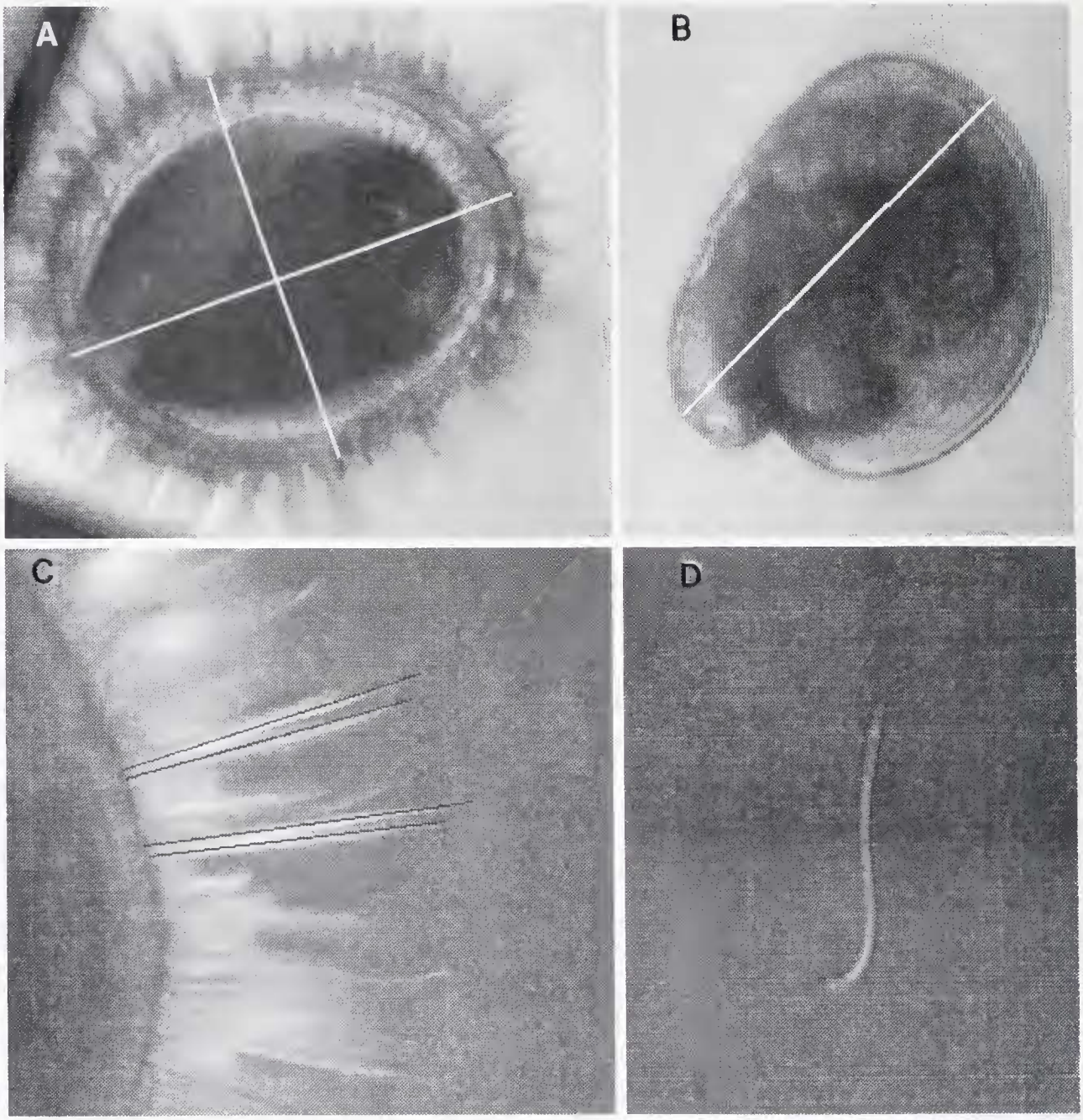

Figure 1. Dimensions of veligers of *Crassostrea gigas* measured from videotaped records. (A) Velar length and width (white lines) of larvae swimming upwards in a cage of nylon mesh. (B) Greatest length of the shell (white line). (C) Lengths of prototrochal cilia in their effective strokes (black lines). (D) Dissected prototrochal cilium (length measured along the curve of the cilium from basal body to cilium tip).

buffered with $CaCO_3$ in seawater. We measured the greatest length of the shell, from the umbo to the shell edge (Fig. 1B).

Finally, the velum of the formalin-fixed larva was torn with tungsten needles, and the slide was searched for intact compound cilia, which were videorecorded through the 10× objective. The longest cilia (Fig. 1D) were measured as a curved line from base to tip. For both methods of measuring cilia, data for cilium lengths were usually the means of five measurements per larva, but sometimes only

three good measurements of a larva's cilia could be obtained from the video-images.

Because videotaped records were inadequate for some measurements on some individual larvae, final sample sizes per jar differed, and the several comparisons of dimensions were therefore made from samples of different sizes.

Jar effects were tested by ANOVA for each measure of the velum or velar cilia, with the jar as a factor and shell length as a covariate in models with and without interaction effects. There were no significant jar effects for any of the four measures of the velum and velar cilia at either high or low concentrations of food. Plots of data for each measure of the velum and velar cilia indicated no differences among jars. Because jar effects were not evident in statistical tests and inspection of plots, veligers from replicate jars were lumped within treatments to increase the degrees of freedom in the analysis of effects of high and low concentrations of food.

Outlying points that might have produced a significant difference based on a few erroneous estimates or abnormal larvae were eliminated from the ANOVAs that tested effects of high and low food levels. These points are the single high value for velum length within the low food treatment in Figure 3, the single low value for length of beating cilium within the high food treatment in Figure 4, and the single low value for length of dissected cilium within the high food treatment in Figure 4.

Effects of food on velar dimensions and cilium lengths were first tested with an ANOVA model with food level as a factor, shell length as a covariate, and the interaction of food level and shell length. In these tests, effects of the interaction were not significant ($P > 0.70$), except for the test for length of beating cilia. Where the interactions were not significant, the F ratio for the interaction was less than $2F_{0.50}$ (Paull, 1950), and we therefore tested for effects of food level and shell length without including their interaction in the ANOVA model.

Results

As expected, larvae at the higher concentration of food grew faster in shell length (Fig. 2) and velar dimensions, and they reached eyespot (Fig. 1B) and pediveliger stages sooner.

More interestingly, larvae reared in a lower concentration of food had velar lobes that were wider, longer, and of greater circumference relative to shell length. When regressions of velar dimensions on shell length were compared, the slopes were similar, but the Y intercept was lower for larvae with the higher concentration of food, indicating smaller velums at a given shell length (Table 1). There was some overlap in the two sets of larvae, however (Fig. 3). The effect of the concentration of food was not significant in an ANOVA model that included the interaction between the factor food and the covariate shell length, but because the interactions were not significant, a simpler model without the interaction was used, and the effect of food was then significant for all three measures of velar size at $P < 0.001$ (ANOVA, df 1, 64).

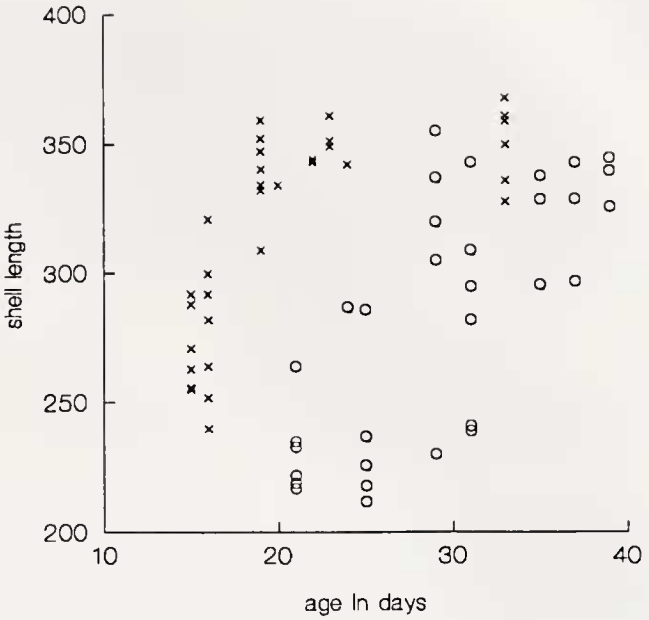

Figure 2. Shell length (μm) *versus* age for sampled veligers at high (x) and low (o) levels of food.

The lengths of prototrochal cilia measured during their effective strokes were greater, relative to shell length, for larvae reared in a lower concentration of food. Because the position of the cilia in their effective strokes could not be judged when larvae were viewed from above, the data are scattered (Fig. 4A). Nevertheless, the effect of food on cilium length was significant at $P < 0.02$ (ANOVA, df 1, 64; natural log transformation; interaction of food level and shell length included in the ANOVA model).

The lengths of prototrochal cilia that had been dissected from the larvae were greater relative to shell length for larvae with a lower concentration of food. Because prototrochal cilium lengths vary, and the position of a cilium on the velum could not be judged after the cilia had been removed, there was considerable scatter and overlap in data for larvae with high and low food (Fig. 4B). The effect of food on cilium length was barely significant at $P < 0.05$ (ANOVA, df = 1, 63; interaction of food and shell length not included in the ANOVA model because of non-significance).

The measured lengths of cilia were greater for dissected cilia than for beating cilia measured during their effective

Table I

Ordinary least squares regression equations for velar dimensions against shell length with all measurements in μm

	SE intercept	SE coefficient	n	r
Velar width *versus* shell length				
High food W = −60.0 + 0.862S	20.7	0.064	30	0.931
Low food W = −23.5 + 0.835S	13.5	0.046	37	0.951
Velar length *versus* shell length				
High food L = −25.1 + 1.005S	23.8	0.073	30	0.933
Low food L = −9.8 + 1.007S	14.9	0.051	37	0.958
Velar circumference *versus* shell length				
High food C = −122.6 + 2.930S	62.7	0.194	30	0.944
Low food C = −49.1 + 2.905S	38.7	0.132	37	0.966

The outlying point for the low food treatment (Fig. 3B) was omitted.

strokes. This difference may result from the inclusion of basal bodies and the curvature of the cilium in measurements of dissected cilia. It is also possible that measurements along the curve of cilia with software for image analysis exaggerated the lengths of dissected cilia.

The increase in cilium length with velar size was similar whether larvae were reared in high or low concentrations of food (Fig. 5).

Discussion

Oyster larvae with less food grew velar lobes that were larger relative to the lengths of their shells (Fig. 3). The velar circumference was relatively greater, and therefore the band of prototrochal cilia that produces the current for feeding and swimming was longer. The prototrochal cilia were longer relative to the shell (Fig. 4) but not longer relative to the velar circumference (Fig. 5). The simplest interpretation of the result is that there is a developmental plasticity in velar growth in response to food.

An alternative interpretation is that these differences result from differential mortality or growth of larvae of different genotypes. This alternative would also imply adaptive differences in form, but would attribute these differences to genetic variation in the population rather than to developmental plasticity. This alternative hypothesis can be rejected when mortality rates are sufficiently low and growth rates uniform, as in a previous study of larval sea urchins (Strathmann *et al.*, 1992), but is a possible explanation of treatment effects in most laboratory experiments on marine larvae, including this one.

If there is additive genetic variation in allocation of materials to larval parts, then measurements of growth of a single part, such as the larval shell, would not necessarily represent patterns of genetic variation in growth of the whole larva. For example, absence of strong selection for rapid larval growth is one interpretation of additive genetic variation in the growth of larval shells (Hilbish *et al.*, 1993). Another possible interpretation is that selection for rapid growth under different food regimes has retarded the loss of heritable variation in growth rate of shells. Abundant food could favor greater allocation to shell growth, and scarce food could favor greater allocation to velar growth. Potential complexities are indicated in Boulding and Hay's (1993) discussion of environmental plasticity and genetic variance for shell shape of intertidal littorines.

Some measurements of the feeding apparatus could be open to interpretations other than differences in growth of shell and velum, but taken together, the measurements indicate differing allocation to the apparatus for capturing food. The velum is extended when it is filled with fluid, and one could argue that hungry larvae simply expanded their velums to a greater extent. It might even be argued that behavioral differences alter the curvature of cilia in their effective strokes; but explanations of this sort cannot be made for cilia dissected from the velum. Because the cilium lengths had about the same relation to velar dimensions for larvae reared with high or low concentrations of food (Fig. 5), the differences in measured sizes indicate differences in growth of the velum as well as of the cilia.

Previous studies of velar function indicate that longer prototrochal cilia and a longer prototrochal band both increase maximum clearance rate (Strathmann and Leise, 1979; Gallager, 1988; Hansen and Ockelmann, 1991). Longer prototrochal cilia also can capture larger particles (Strathmann, 1987b; Hansen, 1991). The differences in larval proportions associated with low or high concentrations of food are therefore those predicted as an adaptive developmental plasticity in response to different concentrations of food. When food is scarce, more growth should be allocated to the apparatus for clearing particles from suspension (prototrochal band, prototrochal cilia). When food is abundant, more growth should be allocated toward

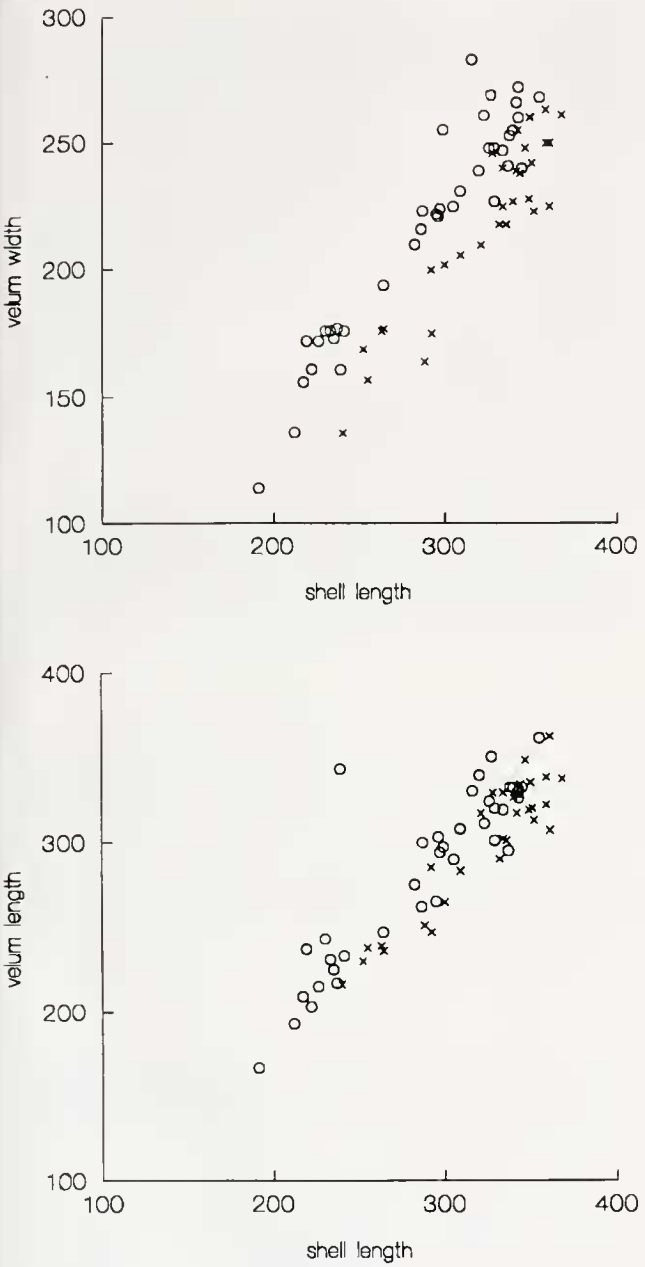

Figure 3. Velar dimensions *versus* shell length with all dimensions in μm. Symbol for veligers with a high concentration of food is x, with a low concentration is o.

development of postlarval structures (shell and associated structures).

The differences in larval proportions in response to food level were not great but were clearly evident. The consequences for clearance rates cannot be accurately calculated because (1) longer cilia capture particles farther from the base of the cilium, but not necessarily in proportion to the increased length of the cilium; (2) longer cilia can have greater angular velocities when there are more simple cilia per compound cilium; and (3) longer cilia may remove larger particles from suspension (Strathmann and Leise, 1979; Strathmann, 1987; Gallager, 1988; Hansen, 1991; Hansen and Ockelmann, 1991). Nevertheless, our experiment provides a minimum estimate of the increases in maximum clearance rates that result from a scarcity of food for veligers that have grown to 300 μm shell length.

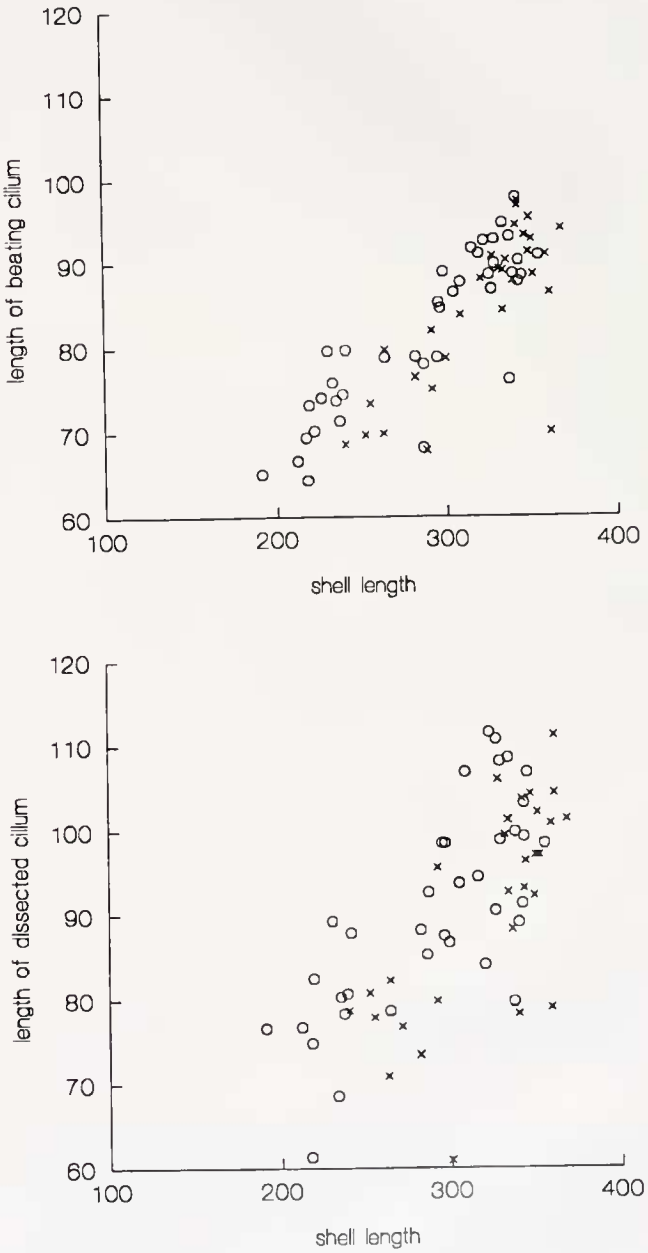

Figure 4. Lengths of prototrochal cilia *versus* shell length with all dimensions in μm. Top: prototrochal cilia in their effective strokes. Bottom: prototrochal cilia dissected from the velum. Symbol for veligers with a high concentration of food is x, with a low concentration is o.

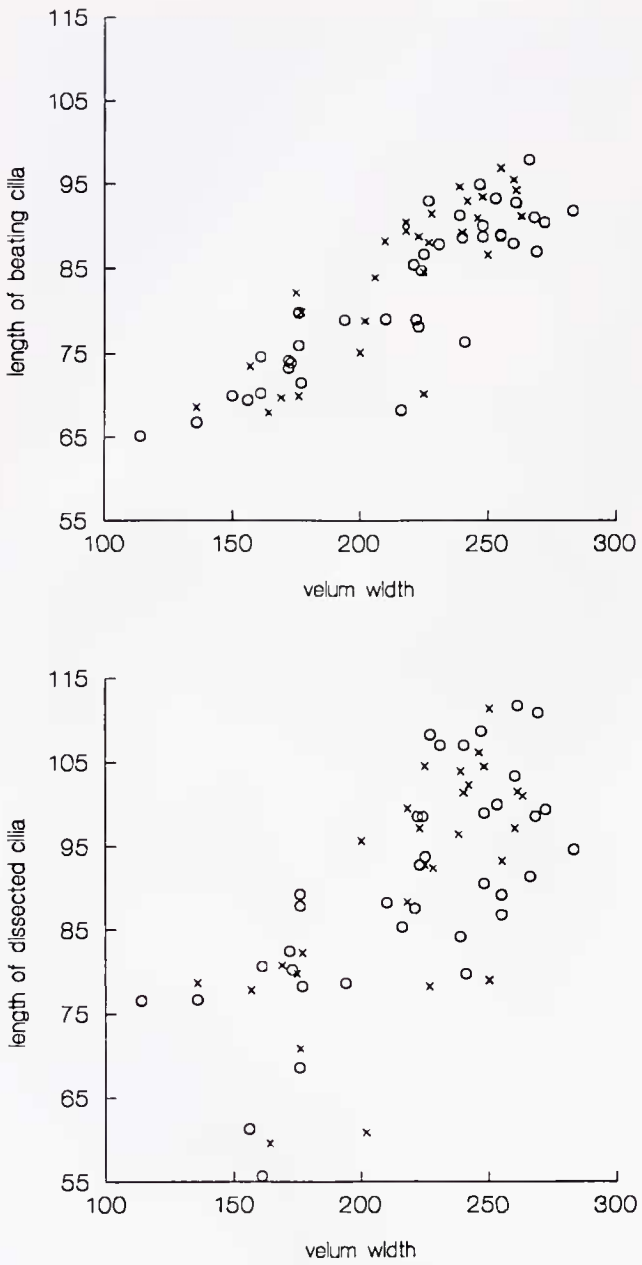

Figure 5. Lengths of prototrochal cilia *versus* velum width with all dimensions in μm. Symbol for veligers with a high concentration of food is x, with a low concentration is o.

Expected velar circumference increased from 756 to 822 μm (Table 1), or 8.7%. Expected length of beating prototrochal cilia increased from 81.3 to 84.2 μm, and expected length of dissected cilia increased from 87.0 to 91.8 μm, increases of 3.6 and 5.5%. At the least, these differences would increase maximum clearance rate by 13 to 15%. Possible increases in the size of food captured and in the angular velocities of cilia could further increase the effect of these size differences on maximum clearance rates. It remains to be determined whether more extreme nutritional conditions produce greater variation in the larvae of *Crassostrea gigas,* or whether greater variation in development occurs in species with larger velar lobes or with a more varied natural supply of food.

Laboratory observations on plutei and veligers suggest that wild larvae that grow at lower concentrations of particulate food should develop a relatively larger capacity for capturing food. This prediction is supported by the few field observations available for plutei (Fenaux *et al.,* 1993; L. Fenaux *et al.,* unpub. obs.). Sampling of planktonic veligers could determine whether variations in their proportions are correlated with concentrations of food in nature.

Not all types of larvae exhibit such a clear separation of ephemeral structures for capturing food and structures needed for settlement or postlarval life. Comparative studies can determine the degree to which developmental plasticity in larval proportions depends on this separation.

Acknowledgments

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Literature Cited

Boidron-Metairon, I. F. 1988. Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. *J. Exp. Mar. Biol. Ecol.* **119:** 31–41.

Boulding, E. G., and T. K. Hay. 1993. Quantitative genetics of shell form of an intertidal snail: constraints on short-term response to selection. *Evolution* **47:** 576–592.

Davis, H. C., and R. R. Guillard. 1958. Relative value of ten genera of micro-organisms as food for oyster and clam larvae. *Fish. Bull. U. S. Fish Wildl. Serv.* **58:** 293–304.

Fenaux, L., M. F. Strathmann, and R. R. Strathmann. 1993. Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei. *Limnol. Oceanogr.* (in press)

Gallager, S. M. 1988. Visual observations of particle manipulation during feeding in larvae of a bivalve mollusc. *Bull. Mar. Sci.* **43:** 344–365.

Hansen, B. 1991. Feeding behaviour in larvae of the opisthobranch *Philine aperta* II. Food size spectra and particle selectivity in relation to larval behaviour and morphology of velar structures. *Mar. Biol.* **111:** 263–270.

Hansen, B., and K. W. Ockelman. 1991. Feeding behaviour in larvae of the opisthobranch *Philine aperta.* I. Growth and functional responses at different developmental stages. *Mar. Biol.* **111:** 255–261.

Hart, M. W. 1991. Particle captures and the method of suspension feeding by echinoderm larvae. *Biol. Bull.* **180:** 12–27.

Hart, M. W., and R. E. Scheibling. 1988. Comparing shapes of echinoplutei using principal components analysis, with an application to larvae of *Strongylocentrotus droebachiensis*. Pp. 277–284 in *Echinoderm Biology*. R. D. Burke, P. V. Mladenov, P. Lambert, and R. L. Parsley, eds. Balkema, Rotterdam.

Hilbish, T. J., E. P. Winn, and P. D. Rawson. 1993. Genetic variation and covariation during larval and juvenile growth in *Mercenaria mercenaria*. *Mar. Biol.* **115:** 97–104.

McEdward, L. R. 1984. Morphometric and metabolic analysis of the growth and form of an echinopluteus. *J. Exp. Mar. Biol. Ecol.* **82:** 259–287.

Nielsen, C. 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zool.* **68:** 205–262.

Pauli, A. E. 1950. On a preliminary test for pooling mean squares in the analysis of variance. *Ann. Math. Statist.* **21:** 539–556.

Rhodes, E. W., and W. S. Landers. 1973. Growth of oyster larvae, *Crassostrea virginica*, of various sizes in different concentrations of the chrysophyte, *Isochrysis galbana*. *Proc. Natl. Shellfish. Assoc.* **63:** 53–59.

Sprung, M. 1984. Physiological energetics of mussel larvae (*Mytilus edulis*). I. Shell growth and biomass. *Mar. Ecol. Prog. Ser.* **17:** 283–293.

Strathmann, M. F. 1987a. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast, Data and Methods for the Study of Eggs, Embryos, and Larvae*. University of Washington Press, Seattle. 670 pp.

Strathmann, R. R. 1978. The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* **32:** 907–914.

Strathmann, R. R. 1987b. Larval feeding. Pp. 465–550 in *Reproduction of Marine Invertebrates*, Vol. 9, *General Aspects: Seeking Unity in Diversity*. A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell, Palo Alto.

Strathmann, R. R., L. Fenaux, and M. F. Strathmann. 1992. Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. *Evolution* **46:** 972–986.

Strathmann, R. R., and E. Leise. 1979. On feeding mechanisms and clearance rates of molluscan veligers. *Biol. Bull.* **157:** 524–535.

Waldock, M. J., and I. A. Nascimento. 1979. The triacylglycerol composition of *Crassostrea gigas* larvae fed on different algal diets. *Mar. Biol. Lett.* **1:** 77–86.